AMENDMENT UNDER 37 C.F.R. § 1.116
EXPEDITED PROCEDURE
GROUP 1655
PATENT APPLICATION

14/B
B. Webb
1/16/01
(NE)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Takahiko, ISHIGURO, et al.

Appln. No.: 09/345,761 Group Art Unit: 1655

Filed: July 01, 1999 Examiner: WILDER, C.

For: METHOD OF ASSAY OF TARGET NUCLEIC ACID

AMENDMENT UNDER 37 C.F.R. § 1.116

ATTN: BOX AF
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

This Amendment is responsive to the Office Action dated July 10, 2000, for which the Examiner set a three-month period for response. A Petition for a three-month extension of time and a check in the amount of $890.00 are submitted herewith, thus making a response due on or before January 10, 2001.

Please amend the above-identified case as follows:

SEQUENCE LISTING:

Please enter the sequence listing submitted herewith.

IN THE CLAIMS:

1. (Amended) A method for assaying a single-stranded RNA in a sample, wherein said RNA contains a specific nucleic acid sequence, said method comprising the following steps:

(1) adding to a reaction vessel containing said single-stranded RNA reagents (A) to (J), wherein said reagents are added to the reaction vessel at constant temperature and one by one, or in combinations of at least two, or all at once: and

(2) after addition of reagents (A) to (I) measuring, at least once, a fluorescent signal in the presence of reagent (J); and

wherein said reagents (A) to (J) are as follows,

(A) a first single-stranded oligo nucleic acid complementary to a sequence 5' of, and adjacent to, the 5' end of said specific nucleic acid sequence,

(B) a second single-stranded oligo DNA complementary to a sequence at the 3' end of said specific nucleic acid sequence,

(C) an RNA-dependent DNA polymerase,

(D) a ribonuclease that degrades RNA in a DNA-RNA double-strand,

(E) deoxyribonucleoside triphosphates,

(F) a third single-stranded oligo DNA having at the 5' end of said oligo DNA the following sequences, in the following order, proceeding in a 5' to 3'

direction with respect to the third single-stranded oligo DNA: 1) a promoter sequence for a DNA-dependent RNA polymerase, 2) an enhancer sequence for said promoter, and 3) a sequence at the 5' end of said specific nucleic acid sequence,

(G) a DNA-dependent DNA polymerase,

(H) a DNA-dependent RNA polymerase,

(I) ribonucleoside triphosphates, and

(J) a fourth single-stranded oligo DNA complementary to said specific nucleic acid sequence, wherein said fourth single-stranded oligo DNA is labeled so that it gives off a measurable fluorescent signal upon hybridization with a nucleic acid containing said specific nucleic acid sequence.

2. (Amended) The method according to Claim 1, wherein the temperature is selected from the range of from 35 to 60°C.

3. (Amended) The method according to Claim 1, wherein the first oligo nucleic acid as the reagent (A) is a DNA, and the method further comprises a step of adding an RNaseH and a subsequent step of deactivating the RNaseH by heating or by addition of an inhibitor prior to addition of the reagent (B).

4. (Amended) The method according to Claim 3, wherein addition of the reagent (A) is followed by simultaneous addition of the reagents (B) to (I), and further by addition of the reagent (J).

B1 cont

5. (Amended) The method according to Claim 3, wherein addition of the reagent (A) is followed by simultaneous addition of the reagents (B) to (J).

6. (Amended) The method according to Claim 1, wherein the first oligo nucleic acid as the reagent (A) is a ribozyme or a DNA enzyme.

Please cancel claims 7 and 8.

9. (Amended) The method according to Claim 1, wherein the enzyme which degrades RNA in a DNA-RNA double strand is the RNA-dependent DNA polymerase as the reagent (D).

B2

10. (Amended) The method according to Claim 1, wherein an enzyme having both an RNA-dependent DNA polymerase activity and a DNA-dependent DNA polymerase activity is used as the reagents (C) and (G) to essentially omit addition of the reagent (C) or the reagent (G).

11. (Amended) The method according to Claim 10, wherein the enzyme is avian myoblastoma virus polymerase.

12. (Amended) The method according to Claim 1, wherein the second and third oligo DNAs as the reagents (B) and (F) are used at concentrations of from 0.02 to 1 μM.

13. (Amended) The method according to Claim 1, wherein the DNA-dependent RNA polymerase as the reagent (H) is at least one enzyme selected from the group consisting of phage SP6 polymerase, phage T3 polymerase, and phage T7 polymerase.

14. (Amended) The method according to Claim 1, wherein the fourth oligo DNA as the reagent (J) is a DNA which is linked to a fluorescent intercalative dye so that the fluorescent intercalative dye changes its fluorescence characteristic upon hybridization of the DNA with another nucleic acid by intercalating into the resulting double strand.

B2 cont

15. (Amended) The method according to Claim 1 or 14, wherein the fourth oligo DNA as the reagent (J) is a DNA which has a 3'end sequence that is not complementary to the specific nucleic acid sequence or has a modified 3' end, and hybridizes to the nucleic acid of Claim 1 having said specific nucleic acid sequence.

16. (Amended) The method according to Claim 1, which further comprises a step of detecting or quantifying the single-stranded RNA in the sample based on the measured fluorescent signal or change in the measured fluorescent signal.

17. (Amended) The method according to Claim 1, wherein all the reagents are chloride-free.

18. (Amended) The method according to Claim 1, wherein step (1) further comprises the addition of an acetate.

B2 cont

19. (Amended) The method according to Claim 18, wherein the acetate is magnesium acetate at a concentration of from 5 to 20 mM or potassium acetate at a concentration of from 50 to 200 mM.

20. (Amended) The method according to Claim 1, wherein step (1) further comprises the addition of sorbitol.

Please cancel claims 21, 22 and 23.

Please add the following new claim 29.

B3

29. The method according to claim 1, wherein the first oligo nucleic acid as the reagent (A) is a DNA.

REMARKS

Claims 1-6, 9-20, and 29 are all the claims pending in the application.

The above-requested amendment to claim 1 reciting an additional reagent (D), which is a ribonuclease that degrades RNA in a DNA-RNA double strand, essentially incorporates the recitation of original claim 7, which has now been canceled. New claim 29, which recites that the first oligo nucleic acid is a DNA merely incorporates that part of original claim 3 which requires that the first oligo nucleic acid be a DNA. New claim 9 does not raise any new issues of patentability because it is broader than original claim 3 and, therefore, would fall with claim 3.

All other amendments to the claims have been made in order to overcome 35 U.S.C. § 112 rejections and/or to make the language of the dependent claims consistent with the incorporation of the recitation of claim 7 into claim 1.

Accordingly, no question of new matter arises and no new issues of patentability are raised. Thus entry of the amendment is respectfully requested.

I. The undersigned thanks the Examiner for correcting the period to respond to the requirement for a sequence listing to coincide with the period to respond to the outstanding Office Action.

II. At page 2, section 1 through page 3, section 4 of the Office Action, the Examiner repeats the telephonic restriction requirement and requests affirmation of Applicants' election.

Accordingly, Applicants affirm their election of the invention of Group I, claims 1-23, without traverse.

III. Prior to discussing the merits of the Office Action, the Examiner's attention is directed the Attachment, which is a schematic diagram of the present invention, as now claimed. As can be seen from the Attachment, the presently claimed method is useful to amplify specific nucleic acid sequences which have non-specific sequences 5' and 3' to the specific nucleic acid sequence. Further, it is not necessary to know the sequence of the 5' end of the specific nucleic acid sequence in order to employ the presently claimed method. This is because the presently claimed method uses a "scissors probe" which is a single-stranded oligo nucleic acid that is complementary to a sequence 5' of, and adjacent to, the 5' end of the specific nucleic acid sequence. This scissors probe allows the non-specific sequence 5' of and adjacent to the 5' end

of the specific nucleic acid sequence to be cleaved, thereby leaving the 5' end of the specific nucleic acid sequence free. Unless the single-stranded RNA has the specific nucleic acid sequence to be amplified at the 5' end before the subsequent amplification cycle is initiated, the amplification cycle does not work well and stops half way.

IV. At pages 3-6, section 6, subsections a to l of the Office Action, the Examiner rejected Claims 1-23 under 35 U.S.C. § 112, second paragraph. The Examiner asserted that these claims are indefinite for failing to specifically point out and distinctly claim the subject matter of the claimed invention.

In particular, the Examiner asserted that the following recited terms and phrases are not clearly defined and/or are confusing. Objections to the language of claims 21-23 have not been addressed, as those claims have been cancelled as non-elected claims in response to the restriction requirement.

(a) In Claim 1, the Examiner believes that the phrase "simple and accurate" uses relative terms. The Examiner suggested deleting the phrase.

Per the Examiner's suggestion, the phrase "simple and accurate" has been deleted from the preamble of Claim 1.

(b) In Claim 1, the Examiner believes that the phrase "almost constant" to define the temperature is a relative term.

Claims 1 and 21 have been amended to delete the term "almost" and to recite "constant temperature" as a condition of adding the reagents. See step (1) of rewritten Claim 1. It is clear

that even the phrase "constant temperature" allows for fluctuation in temperature within experimental error.

(c) In Claim 1, the Examiner believes that the phrase "a step of measuring a fluorescent signal..." is indefinite because it is not clear where, within the method, the step is performed.

Consistent with the Examiner's suggestion, the phrase has been moved to the end of the claimed method.

(d) In Claim 1, the Examiner believes that the phrase "a 3'-end sequence within the specific nucleic acids sequence" is indefinite because "3'-end" cannot be used to describe the location of a sequence "within" the specific nucleic acid sequence.

Claim 1 has been amended to replace the objected to language with "at the 3' end of the specific nucleic acid sequence," consistent with the Examiner's suggestion.

(e) In Claim 1, the Examiner believes that the phrase "a 5'-end sequence within the specific nucleic acids sequence" is indefinite because a "5'-end" cannot be used to describe the location of a sequence "within" the specific nucleic acid sequence.

Claim 1 has been amended to replace the objected to language with "a sequence at the 5' end of said specific nucleic acid sequence," similar to the language suggested by the Examiner with reference to the 3' end.

(f) In Claim 1, the Examiner believes that the phrase "from the 5'-end" is confusing because the phrase can be used to describe either the direction (e.g., proceeding from 5' to 3' end) or location (e.g., at or of the 5' end sequence).

Claim 1 has been amended to replace the objected to language with "proceeding in a 5' to 3' direction with respect to" the recited oligo.

(g) In Claims 18 and 19, the Examiner believes that the phrase "uses an acetate" is indefinite because it is not clear where, in the method, the acetate is used.

Claims 18 and 19 have been amended to recite "wherein step (1) [of Claim 1] further comprises the addition of an acetate." See step (1) of Claim 1 in the set of rewritten amended claims.

(h) In Claim 20, the Examiner believes that the phrase "uses sorbitol" is indefinite because it is not clear where, in the method, the acetate is used.

Claim 20 has been amended to recite "wherein step (1) [of Claim 1] further comprises the addition of sorbital." See step (1) of Claim 1 in the set of rewritten amended claims.

(i) In Claim 1, the Examiner believes that the phrase "at almost constant temperature" is indefinite because it is not clear whether the recited condition is a condition for "a step of adding reagents," as opposed to a condition of the claimed method.

Amended Claim 1 clarifies that all reagents are added at constant temperature.

(j) In Claim 1, the Examiner believes that the phrase "at least the following reagents" is indefinite because it indicates other reagents may be used. The Examiner asserted that since the claims do not define what other reagents may be used the metes and bounds of the claimed invention are not defined.

Applicants disagree with the Examiner's assertion that the metes and bounds of Claim 1 are not defined because the recited phrase indicates that other reagents may be used. Only the essential reagents need to be recited in the claims. This has been done.

(k) The Examiner believes that Claim 15 is not properly dependent from Claims 1 and 14 because the relationship of the recited oligo DNA to the method of Claims 1 and 14 is not recited.

In response, Claim 15 has been amended to additionally recite that the fourth oligo "hybridizes to the nucleic acid of Claim 1 having said specific nucleic acid sequence."

In view of the above, the rejection is believed overcome and withdrawal thereof is respectfully requested.

V. At page 6, section 7, through page 15, section 11 of the Office Action, the Examiner rejected Claims 1-6, 9-13, 16, and 21-23 (now canceled) pursuant to 35 U.S.C. § 103(a). Specifically, the Examiner believes that Claims 1-6, 9-13, 16, and 21-23 are unpatentable over U.S. Patent No. 5,409,818 ("USP '818") to Davey *et al. et al.*(filed June 24, 1988). Moreover, the Examiner believes that Claims 7 and 8 are unpatentable over Davey *et al.* in view of U.S. Patent No. 5,130,238 ("USP '238") to Malek *et al.* (filed August 23, 1989); Claim 14 is unpatentable over Davey *et al.* in view of the publication of Ishiguro *et al.* (published August 2, 1995, Analytical Biochem.); Claims 15 and 20 are unpatentable over Davey *et al.* in view of U.S. Patent No. 5,824,517 ("USP '517") to Cleuziat *et al.* (filed May 16, 1997); and Claims 17, 18, and 19 are unpatentable over Davey *et al.* in view of the publication of Newton (published 1995, PCR, Essential Data).

In order to make a rejection under § 103(a), the Examiner must establish a prima facie case of obviousness. In order to establish such, the Examiner must meet three basic criteria: 1) the references cited by the Examiner must provide some suggestion or motivation to the skilled artisan to modify or combine the teachings of the references to make the claimed invention; 2) the teachings of the cited references must provide some reasonable expectation of success of making the claimed invention; and 3) the cited references must teach, individually or in combination, all the limitations of the claims.

As to Claims 1-6, 9-13, 16, and 21-23, the Examiner believes that Davey *et al.* disclose all elements of the claimed method, except an oligo having an enhancer sequence on the same oligo having the promoter sequence. The Examiner asserted that Davey *et al.* disclose a method of assaying for single stranded RNA containing a specific nucleic acid in a sample, at a "relatively" constant temperature, using the reagents of Claim 1 except for the oligo having the enhancer sequence. The Examiner asserted that it would have been prima facie obvious to a skilled artisan to include an oligo having a promoter sequence and enhancer sequence for the claimed promoter for the benefit of increased transcription because it was known, at the time that applicants' invention was made, that an enhancer sequence greatly increases the efficiency of the promoter. Also, the Examiner asserted that the skilled artisan would have been motivated to modify the detection method of Davey *et al.*, with a reasonable expectation of success, to obtain applicant's invention, including combining the reagents at once or in various combinations and utilizing a constant temperature to increase the quantity of the specific nucleic acid sequence for detection or to increase the purity of the specific nucleic acid. At pages 9 to 11, section 7 of the

Office Action, the Examiner detailed the individual embodiments taught by Davey *et al.* considered as being the same embodiments that Claims 2-6, 9-13, 16, and 21-23 are drawn to.

As to Claims 7 and 8, which were rejected as being unpatentable over Davey *et al.* in view of Malek *et al.*, the Examiner believes that Malek *et al.* disclose a method for assaying a specific nucleic acid sequence using an amplification process including DMSO. Thus, the Examiner asserted that it would have been prima facie obvious, at the time the instant invention was made, to modify the detection method of Davey *et al.* with the teachings of Malek *et al.* to make the claimed invention, because the skilled artisan would have been motivated to include DMSO in the detection method with a reasonable expectation of success. As to Claim 8, the Examiner asserted that Malek *et al.* teach the claimed embodiment of using DMSO at a concentration of from 5 to 20 %.

Claim 14 was rejected as being unpatentable over Davey *et al.* in view of Ishiguro *et al.* The Examiner asserted that, although Davey *et al.* do not teach the use of an intercalative fluorescent dye as a label, such dyes were used in the prior art as exemplified by the teachings of Ishiguro *et al.* Thus, the Examiner asserted that it would have been prima facie obvious, at the time the instant invention was made, to modify the detection method of Davey *et al.* with the teachings of Ishiguro *et al.* to make the claimed invention, because the skilled artisan would have been motivated to use an intercalative fluorescent dye linked to the oligo DNA with a reasonable expectation of success.

Claims 15 and 20 were rejected as being unpatentable over Davey *et al.* in view of Cleuziat *et al.* According to the Examiner, Davey et al do not teach the reagent I oligo having a

sequence not complementary to the sequence at the 3' end of the specific nucleic acid sequence or having a modified 3' end. However, the Examiner asserted that Cleuziat *et al.* disclose a reagent I oligo used as a labeled probe in a method of amplification, wherein the oligo has a modified 3' end and has a label that might be fluorescent or radioactive. Thus, the Examiner asserted that it would have been prima facie obvious, at the time the instant invention was made, to modify the detection method of Davey *et al.* with the teachings of Cleuziat *et al.* to make the claimed invention, because the skilled artisan would have been motivated to use a labeled probe with a modified end with a reasonable expectation of success. As to Claim 20, the Examiner asserted that Cleuziat *et al.* teach the claimed embodiment of using sorbitol.

Claims 17, 18, and 19 were are rejected as being unpatentable over Davey *et al.* in view of Newton. The Examiner stated that Davey *et al.* do not teach that all the reagents are chloride-free. However, the Examiner asserted that in a guide for the polymerase chain reaction ("PCR") Newton discloses amplification buffer containing reagents that are chloride-free. Thus, the Examiner asserted that it would have been prima facie obvious, at the time that the instant invention was made, to modify the detection method of Davey *et al.* with the reaction buffer of Newton to make the claimed invention. The Examiner asserted that the skilled artisan would have been motivated to use a reaction buffer that is chloride-free with a reasonable expectation of success. As to Claim 18, the Examiner cited Newton as teaching the claimed embodiment of using an acetate. As to Claim 19, the Examiner cited Newton as teaching the claimed embodiment of using a magnesium acetate at 10 mM.

For the following reasons, the rejection is respectfully traversed.

The cited references, alone or in any combination, do not provide teachings sufficient for the skilled artisan to make the present invention.

(1) Davey *et al.* (USP5,409,818)

The Examiner alleged that Davey *et al* disclose all elements of the claimed method. However, Davey *et al.* neither disclose nor suggest a first single-stranded oligo nucleic acid as the reagent (A) according to the present invention.

Both in the method of the present invention and the process of Davey *et al.*, in order to amplify the nucleic acid sequence (a characteristic sequence which distinguishes the target nucleic acid from other nucleic acids, which is referred to as the specific nucleic acid sequence in the present invention) it is necessary that the nucleic acid sequence be at the 5' end of the target RNA. Davey *et al.* describe an RNA which functions as the first template as containing at its 5' end a sequence which is sufficiently homologous to that which is at the 3' end of the second primer (from column 9, line 68 to column 10, line 4). That is, the first template contains at its 5' end the specific nucleic acid sequence to be amplified. However, among naturally occurring RNA molecules, some have indefinite sequences at the 5' end. Examples include adeno-associated viruses, HCV (hepatitis C virus), HIV (human immunodeficiency virus) and SRSVs (small round-structured viruses), some have no 5' end due to their circular shape such as HDV (hepatitis D virus), and some do not have a characteristic sequence like the sequence used for determination of the subtype of HCV at the 5' end. The specific nucleic acid sequence in such an RNA can not be amplified by the process Davey *et al.*, without preparing a first template by cutting the RNA so that the first template has the specific nucleic acid sequence at the 5' end. In

this respect, however, Davey *et al.* only disclose that "In an alternative, a template nucleic acid which could function as a first template could be naturally occurring RNA or a RNA fragment which could be generated from a larger RNA molecule by using site specific hydrolysis methods known in the art" (column 10, lines 30-35). Thus, Davey *et al.* say nothing about use of an oligo nucleic acid for the purpose of cutting the target RNA at the 5' end of the specific nucleic acid sequence so that the resulting RNA fragment has the specific nucleic acid sequence located at the 5' end as in the present invention.

Further, the specificity of the site specific hydrolysis methods referred to in Davey *et al.* is not known. That is it is not known whether the site specific hydrolysis methods mentioned in Davey *et al.* cut the RNA so that the specific nucleic acid sequence starts right at the 5' end of the cut RNA. If the target RNA were cut at the middle of the specific nucleic acid sequence, the subsequent reactions would not occur. In contrast, the use of the oligo nucleic acid as the reagent (A) in the present invention has the advantage that it is based on the specificity of the hybridization between the oligo nucleic acid and the target RNA. This is not true of the site specific hydrolysis methods mentioned in Davey *et al.*. According to the present invention, the RNA can not be cut at the middle of the specific nucleic acid sequence because the oligo nucleic acid binds adjacent to the 5' end of the specific nucleic acid sequence in the RNA with sufficient specificity to avoid cutting at incorrect positions.

In addition to the above-mentioned advantage of the present invention, the first single-stranded oligo nucleic acid as the reagent (A) does not inhibit the reactions subsequent to the cleavage of the target RNA such as reverse transcription of RNA, synthesis of cDNA, formation of double-stranded DNA and transcription of double-stranded DNA into RNA. Therefore the

reagent (A) of the present invention can coexist with the other reagents. Therefore, according to the method of the present invention, the assay can be carried out in a sealed vessel once all the reagents and a sample suspected to contain RNA containing the specific nucleic acid sequence are placed in the reaction vessel. When vessels containing genetic amplification products obtained by PCR or the like are opened for determination of the target gene, special care has to be taken to avoid contamination among the samples. Therefore, the method of the present invention which can be carried out in closed vessels is quite useful. Because the site specific hydrolysis methods mentioned by Davey *et al.* seem to use chemical reagents which are detrimental to the other reagents used in the process of Davey *et al.*, the process of Davey *et al.* inevitably involves opening and closing of reaction vessels and therefore can not attain the same effect as the method of the present invention.

In the process of Davey *et al.*, the use of a first and a second primer, which correspond to a second single-stranded oligo DNA (B) and a third single-stranded oligo DNA (F) in the present invention, respectively, prevents erroneous amplification of sequences other than the specific nucleic acid sequence to be amplified and therefore effectively obviates the problem of false positive results often encountered when primers with low specificity are used in amplification of a specific nucleic acid sequence. In the method of the present invention, besides the two single-stranded DNAs (B) and (F) corresponding to the two primers in the process of Davey *et al.*, a first single-stranded oligo nucleic acid as the reagent (A) is used, and the first single-stranded oligo nucleic acid as the reagent (A) lowers the possibility that the wrong RNA will be amplified because only the cleaved RNA is amplified.

In the present invention, the RNA amplification product is detected with a fourth single-stranded oligo DNA labeled with a fluorescent intercalative dye or the like, and its presence in a reaction solution makes it possible to detect the amplified RNA during amplification. On the other hand, Davey *et al.* does not disclose anything about detection of the amplified RNA with a fluorescent intercalative dye-labeled DNA whose presence in a reaction solution makes detection of the amplified RNA possible.

The Examiner should bear in mind that when the first single-stranded oligo nucleic acid as the reagent (A) is a DNAzyme or a ribozyme, it can cut the target RNA at the 5' end of the specific nucleic acid sequence by itself, and when the first single-stranded oligo nucleic acid as the reagent (A) is a DNA with no DNAzyme activity, it may be used with a ribonuclease (which may also have an RNA-dependent DNA polymerase activity like AMV reverse transcriptase) or RNase H (in the case of RNase H, also together with a RNase H inhibitor) (see Claim 3).

The differences between Davey *et al.* and the present invention are especially apparent from the Attachment hereto. Referring to the Attachment, it is clear that reagent (A) of the present invention is not taught or suggested by Davey *et al.*. Reagent (A) (a first single-stranded oligo nucleic acid) according to the present invention does not function as a template, unlike the first template of Davey *et al. et al.*, but rather is used to facilitate cutting the single-stranded RNA in a sample at the 5'-end of the specific nucleic acid sequence to be amplified. Unless the single-stranded RNA has the specific nucleic acid sequence to be amplified at the 5'-end before the subsequent amplification cycle is initiated, the amplification cycle does not work well and stops halfway. Note that the first template of Davey *et al.* corresponds to "the specific nucleic acid sequence to be amplified in the single-stranded RNA in a sample" according to the present

invention, not to the reagent. Davey *et al.* do not disclose a single-stranded oligo nucleic acid which is used to cut the single-stranded RNA before the amplification cycle is started, like the reagent (A) in the present invention.

Further, Davey *et al.* do not teach that the first template can be a single-stranded DNA. The first template of Davey *et al.* has to be a single-stranded RNA (column 3. line 16). Perhaps the Examiner misinterpreted the disclosure in column 11, lines 11-24 of Davey *et al.*. That passage means that a single-stranded RNA, which functions as the first template, or a single-stranded DNA, which functions as the second template could be obtained from "a single-stranded DNA or RNA template" (column 11, lines 11-12 and lines 16-17) which is obtained from "a double-stranded DNA, double-stranded RNA or a DNA-RNA hybrid" (column 11, lines 12-14). Please note that "a single-stranded DNA or RNA template" at lines 11-12 does not mean the first or second template of Davey *et al.* itself; but is equivalent to "the resulting single-stranded DNA or RNA" mentioned at lines 16-17 which could be used to generate the first, second or third template of Davey *et al.*.

In addition, on page 8, lines 6 of the Official Action the Examiner refers to column 43, line 19 of Davey *et al.* for the first oligo nucleotide in the present invention which is a DNA recited in Claim 3. However, the disclosure in column 6, line 19 relates to the first primer of Davey *et al.* which has at its 3' end a sequence which is sufficiently complementary to the 3' end of the first template, whereas the first oligo nucleic acid (reagent (A)) in the present invention is complementary to a sequence neighboring the 5' end of the specific nucleic acid sequence in the single-stranded RNA. Thus, Davey *et al.* do not disclose the reagent (A) in the

present invention which is used to facilitate cutting a single-stranded RNA in a sample at the 5'-end of the specific nucleic acid sequence to be amplified in the single-stranded RNA.

As pretreatment of an RNA for preparation of a first template, Davey *et al.* only mention site specific hydrolysis in column 10, lines 30-35.

However, Davey *et al.* disclose site specific hydrolysis only as a general method for fragmentation of RNA. The purpose of using reagent (A) to facilitate RNA cleavage in the present invention is to enable amplification of a specific sequence which is present somewhere other than at the 5' end of the RNA to be detected in a sample by cleaving the RNA at the 5' end of the of the specific sequence so that the 5' end of the specific sequence becomes the 5' end of the resulting RNA fragment. This is important, because it is impossible to amplify such a specific sequence in the middle of an RNA when the RNA is kept intact. Davey *et al.* does not suggest the purpose for using reagent (A) in the present invention, not to mention the effect of using reagent (A).

It should be understood that amplification of a specific sequence which is present somewhere other than at the 5' end of an RNA can be attained only by the use of reagent (A). In the present invention, in order to amplify a specific sequence which is present somewhere other than at the 5' end of an RNA, not only reagent (A), but also reagent (B) which is complementary to a sequence at the 3' end of the specific sequence and the reagent (F) which has at the 3' end a sequence at the 5' end of the specific sequence are used are in combination. Davey *et al.* does not teach or suggest the combined use of the mutually related three reagents

or teach that the combined use of these reagents realizes more sequence-specific detection of a specific nucleic acid sequence.

According to the present invention, the cleavage of a single-stranded RNA using reagent (A) can take place even in the presence of the other reagents (B) to (J). Therefore, the method of the present invention can be performed by just putting all the reagents recited in Claim 1 into a reaction vessel.

(2) Malek *et al* (USP 5,120,238)

Malek *et al.* disclose assaying a specific nucleic acid sequence using an amplification process in which DMSO is included in the amplification reaction medium. However, Malek *et al.* do not disclose anything about the first single-stranded oligo nucleic acid as the reagent (A) in the present invention. Therefore, the combination of Davey *et al.* with Malek *et al.* do not result in the present invention, even as claimed in Claim 1

(3) Ishiguro *et al.*

Ishiguro *et al.* disclose a method of assaying viral RNA using PCR in the presence of a fluorescent intercalative dye. However, the amplification product in the method of Ishiguro is a double-stranded DNA while the amplification product in the method of the present invention is an RNA.

Further, Ishiguro only discloses that the presence of a fluorescent intercalative dye does not interfere with amplification by PCR. Ishiguro *et al.* do not mention anything about the amplification method using the first single-stranded oligo nucleic acid as the reagent (A) in the

present invention. The present inventors were the first to demonstrate that the claimed amplification process proceeds in the presence of a fluorescent intercalative dye-labeled single-stranded DNA as the reagent (J).

(4) Cleuziat *et al.* (USP 5,824,517) and Newton

Cleuziat *et al.* and Newton merely disclose elements claimed in some dependent claims and do not suggest use of the first single-stranded oligo nucleic acid the preset invention or use of the fourth single-stranded oligo DNA labeled with a fluorescent intercalative dye for detection of the amplified RNA.

CONCLUSION

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

Applicant hereby petitions for any extension of time which may be required to maintain the pendency of this case, and any required fee, except for the Issue Fee, for such extension is to be charged to Deposit Account No. 19-4880.

Respectfully submitted,

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